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14. ABSTRACT

Our idea is to apply a series of novel techniques to identify the reagents needed to move imaging technology forward into the clinic. While molecular imaging strategies are now approaching the resolution required to detect ovarian cancer in an early curable stage, specific imaging probes are not currently available and are urgently needed to realize the potential of imaging for ovarian cancer early detection. To address this challenge we are undertaking a comprehensive proteomic analysis of the cell surface membrane of ovarian cancer cells. We have completed a survey of the cell surface glycoproteome of OVCAR3 cells using a novel biochemical labeling method that allows for highly selective capture and internal validation of candidate peptides and proteins by LC-MS/MS. To date 209 proteins have been identified. We are currently annotating these proteins as potential targets for molecular imaging probes. Several promising candidates have been identified.

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Targeting Cell Surface Proteins in Molecular Photoacoustic Imaging to Detect Ovarian Cancer Early

Charles W Drescher, MD, Principle Investigator

Table of Contents

Page

INTRODUCTION

Our idea is to apply a series of novel techniques to identify the reagents needed to move imaging technology forward into the clinic. While molecular imaging strategies are now approaching the resolution required to detect ovarian cancer in an early curable stage, specific imaging probes are not currently available and are urgently needed to realize the potential of imaging for ovarian cancer early detection. To address this challenge we will conduct for the first time a comprehensive, comparative survey of the surface proteome of serous ovarian cancer and human ovarian surface epithelial cells in order to select and validate ovarian cancer specific surface proteins for use as targets in molecular photoacoustic imaging, an especially promising imaging strategy for ovarian cancer early detection.

BODY

Shortly after the onset of the funding period we began testing a novel method for capture of N-linked glycoproteins from the surface of intact viable cells. The method is highly efficient; when applied to lymphocyte cell lines greater than 95% of identified proteins were confirmed as from the cell surface[1]. The approach involves gentle, covalent chemical labeling of oxidized carbohydrate-containing proteins on live cells using the bi-functional linker molecule, biocytin hydrazide (BH), affinity enrichment of BH-labeled peptides and specific enzymatic peptide release followed by peptide and protein identification using LC-MS/MS. Selective identification of glycosylated peptides is confirmed via identification of NSX/T motifs and a specific mass shift induced by the enzymatic release. Quantitative comparative analysis is possible by incorporation of isotopic labels using succinic anhydride[2]. In light of the striking selectivity we sought to test and validate this approach using OVCAR3 cells. Results from these experiments are described below and demonstrate reproducible and highly selective isolation of surface glycoproteins from ovarian cancer cells; to date a total of 209 candidate ovarian cancer cell surface glycoproteins have been identified. Based on our experience we believe this biochemical capture method has several advantages over the N-Sulfo-NHS-SS-BIOTIN labeling we employed in our early studies and described in our proposal. Consequently, we are planning to substitute the N-linked glycocapture method for profiling of serous ovarian cancer cells from ascites and control materials. The overall scope of work and project goals remain unchanged. Progress on specific project tasks based on profiling of OVCAR3 cells using the N-glycocapture method are outlined below.

Task 1 (Months 1-2): *Obtain and establish culture conditions for ovarian cancer cells and HOSE cells*

Ovarian cancer cells were isolated from ascites collected at primary surgery from patients with serous ovarian cancer by centrifugation. Pelleted cells were then treated with RBC lysis solution to remove contaminating red blood cells. The remaining cells were assessed for viability and percent tumor content. If necessary, cells were further purified with a percoll density gradient to remove any non-tumor cells or with a ficoll gradient to remove lymphocytes, dead cells and debris. The remaining tumor cells were then viably frozen. A summary of processed ascites samples available and suitable for the proposed work is summarized in **Table 1**. We have analyzed aliquots from all samples to confirm purity and viability which ranged from 50-90%.

Table 1: Ascites samples processed to obtain serous ovarian cancer cells.

Cell Specimen IDs	Patient Histology	Stage III/IV	Sample Quality	# Vials Frozen	# Cells/Vial
394288	Papillary Serous	Yes	Excellent	100	5.00E+09
400964	Papillary Serous	Yes	Excellent	26	5.00E+07
400119	Papillary Serous	Yes	Excellent	25	5.00E+07
401109	Serous	Yes	Excellent	30	5.00E+07
429546	Serous	Yes	Excellent	25	5.00E+07
476414	Serous with some clear cell features (TIC also)	Yes	Excellent	100	5.00E+07

We have frozen aliquots of immortalized HOSE cells for control material. Given the uncertainty regarding the cell of origin for serous ovarian cancer we evaluated fallopian tube epithelial cells as a potential

source of controls. However, upon culturing these cells appeared contaminated with fibroblast and/or other stromal cells and were not suitable for additional studies.

Task 2 (Months 2-4): *Isolate proteins from cell surface and whole cell lysates of case and control cells*

We elected to evaluate the N-linked glycoprotein capture method using OVAR3 cells so that we could compare with prior results using the N-Sulfo-NHS-SS-BIOTIN labeling method. Control cells were not used in these experiments. Following initial experiments to optimize processing conditions for ovarian cancer cells, roughly 8 $X10^7$ OVCAR3 cells were gently oxidized and then BH-labeled. The cells were lysed in the presence of protease inhibitors and then homogenized using a dounce homogenizer. The membrane fraction was isolated and then sonicated prior to trypsin digestion of the proteins. At this point digestion and labeling was confirmed via western blot. Digested peptides were then incubated with streptavidin beads. A dot blot was used to confirm that all peptides were successfully bound to beads and, if necessary, additional beads were added. Beads were then washed and treated with PNGaseF to remove the peptides from the beads. Glycopeptides were washed, dried down via speedvac and cleaned up via SCX. Captured peptides were analyzed using mass spectrometry.

Task 3 (Months 4-6): *Mass spectrometry interrogation of protein fractions from case-control pools*
and
Task 4 (Months 6-10): *Signal processing and production of analytic data set to identify candidate markers*

Single fractions of undiluted eluate and eulate diluted 1:10 were interrogated by LC-MS/MS in a LTQ-ORBITRAP mass spectrometer (Thermo-Finnigan) coupled to a nanoflow chromatography system (Eksigent) over a 90 minute linear gradient. Acquired tandem mass spectra were searched using X!Tandem against a combined database of the human (v. 3.44) and bovine (v. 3.30) IPI database released on May, 2008. Peptide confidence was determined using PeptideProphet, and only those peptides achieving probability greater than 0.95 accuracy and which were not bovine-specific were submitted to ProteinProphet for grouping into proteins or assemblies of proteins. We next filtered the protein list to omit proteins derived from fetal bovine serum (FBS) (proteins from the media). Only protein groups that contain at least one peptide specific to human are retained (e.g., we eliminated all groups that were bovine-specific or all peptide evidences are ambiguous between bovine and human). Each protein group was then assigned a spectral count, a quantitative value that counts the number of total times a peptide from that protein or protein group is observed. The spectral count has been shown to be proportional to the relative abundance of the protein in the sample.

A total of 1339 peptides were identified across both fractions; 1301 peptides contained the modified NSX/T motif. These peptides mapped to 187 proteins, 154 of which contained at least one NXS/T motif. One hundred fifty-three of the 154 proteins were classified as cell surface proteins using standard bio-informatics techniques (TMHMM, GO) and/or literature review. Analysis of the individual fractions demonstrates that the dilute fraction contained roughly 50% fewer peptides (415 vs. 924) but yielded roughly 20% more proteins (155 vs. 126). The list of identified proteins includes a number of established ovarian cancer cell surface markers including MUC16, mesothelin, CD276 and CD47 (aka OVA3). To the best of our knowledge this is the first unbiased protein discovery effort to identify MUC16 as an ovarian cancer cell surface protein. The approach is far more efficient than the Sulfo-NHS-SS-BIOTIN labeling method where in a prior experiment only 84 of 758 (11%) of proteins captured from OVCAR3 cells could be annotated to the cell surface. We evaluated the reproducibility of the N-glycocapture method by profiling additional OVCAR3 cells in a second experiment. A total of 125 candidate cell surface proteins were identified including 101 proteins identified in the earlier experiment. Combined across both experiments a total of 209 high confidence cell surface proteins have been identified (see **Appendix A,** bold font denotes newly identified in second experiment). Based on these encouraging results we are planning to use the N-linked glycocapture method to profile the serous ovarian cancer cells isolated from ascites and control material. This work is scheduled to start in August, 2011.

Task 5 (Months 10-12): *Rank candidate ovarian surface proteins through integration with other datasets*

The 209 candidate proteins were annotated using bio-informatics methods and datasets available to our group. Specifically, we determined if they had been identified in prior proteomic profiling studies using OVCAR3 and ES2 ovarian cancer cell lines and evaluated them for evidence of secretion in ascites fluid collected from patients with ovarian cancer and benign tumors (as a negative control) and plasma from healthy individuals. A gene expression dataset was used to determine the relative expression of each candidate in 35 normal tissue types. The Human Protein Atlas database (www.proteinatlas.org) was used to further annotate candidates based on the distribution and intensity of protein level expression in ovarian cancer and normal tissues. The Human Protein Atlas is a publicly available database of high-resolution images showing the spatial distribution of proteins in 46 different normal human tissues and 20 different cancer types, as well as 47 different human cell lines. The data are released together with application-specific validation performed for each antibody. One hundred fifty-one of 209 candidates were represented in The Human Protein Atlas database. For these candidates we downloaded the following information: a) the overall proportion of ovarian cancer tissues stained, b) ovarian cancer staining intensity (weak, intermediate or strong) and c) number of normal tissues that expressed the protein. We are currently mining this dataset to rank candidate ovarian surface proteins as targets for molecular imaging probes.

An ideal target for an ovarian cancer molecular imaging probe includes proteins that are highly and specifically expressed on the surface of ovarian cancer cells, expressed lowly or not at all on normal cells and of low abundance in the plasma of healthy patients and patients with cancer. Below we provide information on 3 interesting candidates including images downloaded from The Human Protein Atlas database (**Figure 1**). Importantly, images from ovarian cancer tissues demonstrate distinct cell-surface expression of candidate markers and confirm the robustness of the glycocapture method.

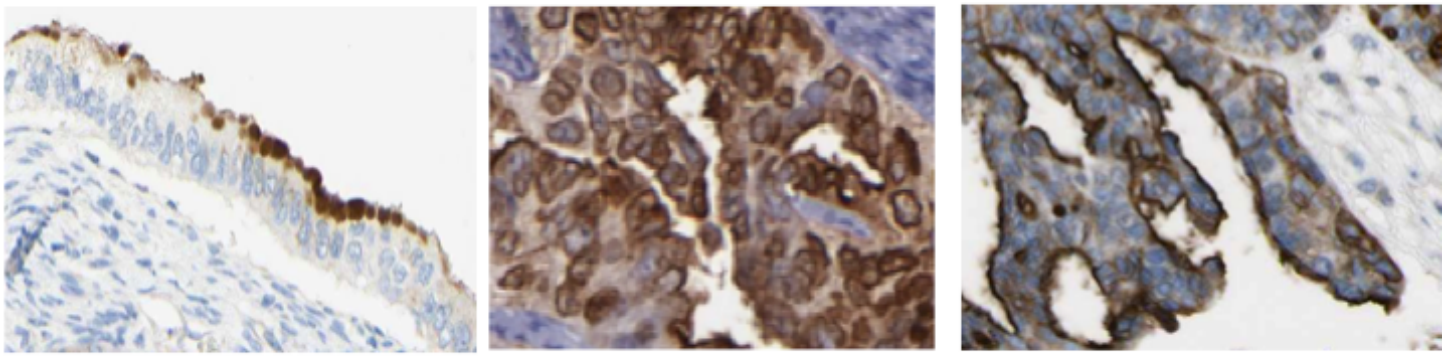
SLC46A1 is a transmembrane proton-coupled folate transporter protein that facilitates the movement of folate and antifolate substrates across cell membranes optimally in acidic pH environments. This protein falso functions as a heme transporter in duodenal enterocytes and potentially in other tissues like liver and kidney. The Human Protein Atlas demonstrates diffuse, weak membrane staining in 58% of ovarian cancer samples (**Figure 1a**). In normal tissues the gastric mucosa, basal cells in ductus seminiferus and occasional airway epithelial cells showed moderate cytoplasmic positivity. Weak cytoplasmic positivity was observed in intestinal glands while most remaining cells were negative. We did not identify secreted forms of SLC46A1 in ascites or normal plasma.

MFGE8 is a protein that plays an important role in the maintenance of intestinal epithelial homeostasis and the promotion of mucosal healing and promotes VEGF-dependent neovascularization. It contributes to phagocytic removal of apoptotic cells in many tissues and is a specific ligand for the alpha-v/beta-3 and alpha-v/beta-5 receptors. The Human Protein Atlas demonstrates moderate to strong membrane/cytoplasmic staining in 61% of ovarian cancers (**Figure 1b**) and cytoplasmic expression in trophoblasts, large arteries and subset of bone marrow poietic cells. We did not find evidence for secreted forms.

SUSD2 is a single-pass Type I membrane protein of unknown function. The Human Protein Atlas database demonstrates evidence of membrane expression in 100% of ovarian cancers (**Figure 1c**); however, cytoplasmic and membrane staining is also noted in many normal tissues with strongest staining noted in alveolar cells and renal tubules. Weak staining was noted in normal ovary. SUSD2 was noted in low abundance in ascites from cancer patients and was not detected in ascites from benign tumors or normal plasma.

Figure 1. Protein level expression of candidate markers in ovarian cancer tissues*

A) SLC46A1 B)MFGE8 C) SUSD2



* Representative section

KEY RESEARCH ACCOMPLISHMENTS

- We have completed a proteomic survey of the surface glycoproteome of OVCAR3 cells
- Several candidate targets for ovarian cancer molecular imaging probes have been identified

REPORTABLE OUTCOMES

None to date

CONCLUSION

Biocytin hydrazide labeling of gently oxidized living cells is a highly efficient method for capture of ovarian cancer cell surface gylocoproteins for subsequent interrogation by LC-MS/MS. A survey of the glycoprotein of OVCAR3 cells has identified over 200 proteins, some of which have potential as diagnostic and/or therapeutic targets.

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1. Wollscheid B, Bausch-Fluck D, Henderson C, O'Brien R, Bibel M, Schiess R, Aebersold R, Watts JD. Mass-spectrometric identification and relative quantification of N-linked cell surface glycoproteins. Nat Biotechnol. 2009 Apr;27(4):378-86. Epub 2009 Apr 6.

2. Zhang H, Li XJ, Martin DB, Aebersold R. Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. Nat Biotechnol. 2003 Jun;21(6):660-6. Epub 2003 May 18.

index	MasterGroup.ID	symbol	protein	labeled	is_membrane	TMHMM	SOSUI	is_on_surface
281	OVCAR3281	MUC16	h_IPI00103552;h_	Yes	Yes	Yes	MEMBRANE	Yes
310	OVCAR3310	MME	h_IPI00247063	Yes	Yes	Yes	MEMBRANE	Yes
226	OVCAR3226	EGFR	h_IPI00018274;h_	Yes	Yes	Yes	MEMBRANE	Yes
333	OVCAR3333	ERBB2	h_IPI00300384;h_	Yes	Yes	Yes	MEMBRANE	Yes
179	OVCAR3179	CD44	h_IPI00002541;h_	Yes	Yes	Yes	MEMBRANE	Yes
82	OVCAR382	NCAM1	b_IPI00702710;h_	Yes	Yes	Yes	MEMBRANE	Yes
242	OVCAR3242	TFRC	h_IPI00022462	Yes	Yes	Yes	MEMBRANE	Yes
371	OVCAR3371	TSHR	h_IPI00744312	Yes	Yes	Yes	MEMBRANE	Yes
206	OVCAR3206	CD59	h_IPI00011302	Yes	Yes	No	MEMBRANE	Yes
213	OVCAR3213	ADAM10	h_IPI00013897;h_	Yes	Yes	Yes	MEMBRANE	Yes
17	OVCAR317	ALCAM	b_IPI00687372;h_	Yes	Yes	Yes	MEMBRANE	Yes
251	OVCAR3251	MSLN	h_IPI00025110;h_	Yes	Yes	Yes	MEMBRANE	Yes
308	OVCAR3308	ANPEP	h_IPI00221224	Yes	Yes	Yes	MEMBRANE	Yes
259	OVCAR3259	ITGB4	h_IPI00027422;h_	Yes	Yes	Yes	MEMBRANE	Yes
151	OVCAR3151	BSG	b_IPI00717356;h_	Yes	Yes	Yes	MEMBRANE	Yes
350	OVCAR3350	BSG	h_IPI00394876	Yes	No	Yes	MEMBRANE	Yes
261	OVCAR3261	ITGAV	h_IPI00027505;h_	Yes	Yes	Yes	MEMBRANE	Yes
307	OVCAR3307	ITGB3	h_IPI00220350;h_	Yes	Yes	Yes	MEMBRANE	Yes
233	OVCAR3233	CD82	h_IPI00020446;h_	Yes	Yes	Yes	MEMBRANE	Yes
177	OVCAR3177	MFGE8	h_IPI00002236	Yes	No	No		Yes
220	**OVCAR3220**	**SLC1A4**	**h_IPI00015476**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
306	OVCAR3306	SLC2A1	h_IPI00220194;h_	Yes	Yes	Yes	MEMBRANE	Yes
114	OVCAR3114	SIRPA;SIRPB1	b_IPI00708771;b_	Yes	Yes	Yes	MEMBRANE	Yes
369	OVCAR3369	ITGB1	h_IPI00645194	Yes	Yes	Yes	MEMBRANE	Yes
184	OVCAR3184	LAMP1	h_IPI00004503;h_	Yes	Yes	Yes	MEMBRANE	Yes
205	OVCAR3205	IFNGR1	h_IPI00010808;h_	Yes	Yes	Yes	MEMBRANE	Yes
272	**OVCAR3272**	**TNC**	**h_IPI00031008;h_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
262	OVCAR3262	IL1R1	h_IPI00027508	Yes	Yes	Yes	MEMBRANE	Yes
322	OVCAR3322	F2R	h_IPI00296869	Yes	Yes	Yes	MEMBRANE	Yes
204	OVCAR3204	ITGA6	h_IPI00010697;h_	Yes	Yes	Yes	MEMBRANE	Yes
246	OVCAR3246	NEO1	h_IPI00023814;h_	Yes	Yes	Yes	MEMBRANE	Yes
160	OVCAR3160	CLCN2	b_IPI00725790;h_	Yes	No	Yes	MEMBRANE	Yes
202	OVCAR3202	F3	h_IPI00010338;h_	Yes	Yes	Yes	MEMBRANE	Yes
248	OVCAR3248	HSPG2	h_IPI00024284	Yes	No	No	MEMBRANE	Yes
258	**OVCAR3258**	**IGF1R**	**h_IPI00027232**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
116	OVCAR3116	JAG1	b_IPI00709263;h_	Yes	Yes	Yes	MEMBRANE	Yes

227	OVCAR3227	IGFBP3	h_IPI00018305;h_	Yes	No	No		Yes
78	OVCAR378	EPHA2	b_IPI00700599;h_	Yes	Yes	Yes	MEMBRANE	Yes
243	OVCAR3243	SORL1	h_IPI00022608	Yes	Yes	Yes	MEMBRANE	Yes
93	OVCAR393	SLC46A1	b_IPI00705235;b_	Yes	Yes	Yes	MEMBRANE	Yes
311	OVCAR3311	RTN4R	h_IPI00289204;h_	Yes	Yes	No		Yes
13	OVCAR313	EFNA5	b_IPI00686957;h_	Yes	Yes	No	MEMBRANE	Yes
43	OVCAR343	EPHB2	b_IPI00693216;b_	Yes	No	Yes	MEMBRANE	Yes
364	OVCAR3364	HLA-B;HLA-C;MICA	h_IPI00472162;h_	Yes	Yes	Yes	MEMBRANE	Yes
363	OVCAR3363	HLA-B;HLA-C;MICA	h_IPI00472138;h_	Yes	Yes	Yes	MEMBRANE	Yes
185	OVCAR3185	HLA-B;HLA-C;MICA	h_IPI00004657;h_	Yes	Yes	Yes	MEMBRANE	Yes
283	OVCAR3283	HLA-B;HLA-C;MICA	h_IPI00107380;h_	Yes	Yes	Yes	MEMBRANE	Yes
296	**OVCAR3296**	**SLC22A4;SLC22A5**	**h_IPI00171334;h_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
300	OVCAR3300	CD47	h_IPI00216514;h_	Yes	Yes	Yes	MEMBRANE	Yes
212	OVCAR3212	ITGA2	h_IPI00013744	Yes	Yes	Yes	MEMBRANE	Yes
58	OVCAR358	PLXND1	b_IPI00696453;b_	Yes	Yes	Yes	MEMBRANE	Yes
236	**OVCAR3236**	**NCSTN**	**h_IPI00021983;h_**	**Yes**	**Yes**	**Yes**		**Yes**
92	**OVCAR392**	**ATP1A2;ATP1A1;A**	**b_IPI00705159;b_**	**No**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
234	**OVCAR3234**	**SLC4A7**	**h_IPI00021058;h_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
315	OVCAR3315	PODXL2	h_IPI00291300;h_	Yes	Yes	Yes	MEMBRANE	Yes
171	OVCAR3171	ERBB3	b_IPI00867170;h_	Yes	Yes	Yes	MEMBRANE	Yes
50	OVCAR350	FGFRL1	b_IPI00695109;h_	Yes	No	Yes	MEMBRANE	Yes
305	OVCAR3305	ICOSLG	h_IPI00219131;h_	Yes	Yes	Yes	MEMBRANE	Yes
190	OVCAR3190	LTBR	h_IPI00006097	Yes	No	Yes	MEMBRANE	Yes
152	OVCAR3152	FGFR1	b_IPI00717840;h_	Yes	Yes	Yes	MEMBRANE	Yes
159	OVCAR3159	FAT2	b_IPI00725027;h_	Yes	Yes	Yes	MEMBRANE	Yes
30	OVCAR330	RYK	b_IPI00690147;h_	Yes	Yes	Yes	MEMBRANE	Yes
144	OVCAR3144	CADM1	b_IPI00714476;h_	Yes	Yes	Yes	MEMBRANE	Yes
106	**OVCAR3106**	**ELFN2**	**b_IPI00707086;h_**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
9	OVCAR39	ECE1	b_IPI00686696;b_	Yes	No	Yes	MEMBRANE	Yes
231	OVCAR3231	EFNB3	h_IPI00019501	Yes	Yes	Yes	MEMBRANE	Yes
266	OVCAR3266	ITGB5	h_IPI00029741	Yes	Yes	Yes	MEMBRANE	Yes
331	**OVCAR3331**	**PODXL**	**h_IPI00299116;h_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
194	OVCAR3194	ICAM1	h_IPI00008494;h_	Yes	Yes	Yes	MEMBRANE	Yes
286	OVCAR3286	CD55	h_IPI00152418;h_	Yes	Yes	No		Yes
340	OVCAR3340	ITGA5	h_IPI00306604	Yes	Yes	Yes	MEMBRANE	Yes
136	OVCAR3136	CSPG4	b_IPI00713597;h_	Yes	Yes	Yes	MEMBRANE	Yes
380	OVCAR3380	PLXNB2	h_IPI00853369	Yes	No	No	MEMBRANE	Yes

294	OVCAR3294	PTK7	h_IPI00168813;h_	Yes	Yes	Yes	MEMBRANE	Yes
316	OVCAR3316	SLC44A2	h_IPI00293074;h_	Yes	No	Yes	MEMBRANE	Yes
48	OVCAR348	CXADR	b_IPI00694859;h_	Yes	Yes	Yes	MEMBRANE	Yes
228	OVCAR3228	TNFRSF1A	h_IPI00018880;h_	Yes	Yes	Yes	MEMBRANE	Yes
235	OVCAR3235	SUSD2	h_IPI00021302	Yes	Yes	Yes	MEMBRANE	Yes
196	**OVCAR3196**	**IFITM2**	**h_IPI00008922**	**No**	**No**	**Yes**	**MEMBRANE**	**Yes**
142	OVCAR3142	DSG2	b_IPI00714378;h_	Yes	Yes	Yes	MEMBRANE	Yes
60	OVCAR360	L1CAM	b_IPI00696555;b_	Yes	Yes	Yes	MEMBRANE	Yes
313	OVCAR3313	PTPRJ	h_IPI00290328;h_	Yes	Yes	Yes	MEMBRANE	Yes
183	OVCAR3183	TNFRSF21	h_IPI00004413	Yes	No	Yes	MEMBRANE	Yes
303	**OVCAR3303**	**SORT1**	**h_IPI00217882**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
35	OVCAR335	CDH6	b_IPI00691400;h_	Yes	Yes	Yes	MEMBRANE	Yes
357	OVCAR3357	ALPL	h_IPI00419916	Yes	Yes	No	MEMBRANE	Yes
175	OVCAR3175	TSPAN13	h_IPI00000735	Yes	Yes	Yes	MEMBRANE	Yes
12	OVCAR312	PLXNA1	b_IPI00686867;b_	Yes	No	Yes	MEMBRANE	Yes
52	OVCAR352	ACVR1	b_IPI00695437;b_	Yes	Yes	Yes	MEMBRANE	Yes
237	OVCAR3237	IL18R1	h_IPI00021999	Yes	Yes	Yes	MEMBRANE	Yes
271	OVCAR3271	MST1R	h_IPI00030273;h_	Yes	Yes	Yes		Yes
23	OVCAR323	EPHB3	b_IPI00689224;b_	Yes	Yes	Yes	MEMBRANE	Yes
73	OVCAR373	CACNA2D1	b_IPI00699719;h_	Yes	Yes	No	MEMBRANE	Yes
65	OVCAR365	ATRN	b_IPI00697240;b_	Yes	Yes	Yes	MEMBRANE	Yes
188	OVCAR3188	EFNB2	h_IPI00005126	Yes	Yes	Yes	MEMBRANE	Yes
230	OVCAR3230	CD276	h_IPI00019275;h_	Yes	Yes	Yes	MEMBRANE	Yes
288	OVCAR3288	CD109	h_IPI00152540;h_	Yes	Yes	No	MEMBRANE	Yes
222	OVCAR3222	C1orf159	h_IPI00016627;h_	Yes	No	Yes	MEMBRANE	Yes
299	OVCAR3299	CD63	h_IPI00215998;h_	Yes	Yes	Yes	MEMBRANE	Yes
198	OVCAR3198	TPBG	h_IPI00009111	Yes	Yes	Yes	MEMBRANE	Yes
167	**OVCAR3167**	**GOLM1**	**b_IPI00840440;h_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
265	OVCAR3265	ADAM17	h_IPI00029606;h_	Yes	Yes	Yes	MEMBRANE	Yes
155	OVCAR3155	PVRL4	b_IPI00718186;h_	Yes	Yes	Yes	MEMBRANE	Yes
312	OVCAR3312	CDCP1	h_IPI00290039;h_	Yes	Yes	Yes	MEMBRANE	Yes
210	**OVCAR3210**	**SPINT2**	**h_IPI00011662**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
351	OVCAR3351	VASN	h_IPI00395488	Yes	No	Yes	MEMBRANE	Yes
326	OVCAR3326	HEG1	h_IPI00297263;h_	Yes	No	Yes	MEMBRANE	Yes
354	OVCAR3354	ITFG3	h_IPI00396658;h_	Yes	No	Yes	MEMBRANE	Yes
127	**OVCAR3127**	**IGSF8**	**b_IPI00712155;h_**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
133	OVCAR3133	UNC5C;UNC5B	b_IPI00712941;b_	Yes	No	Yes	MEMBRANE	Yes

221	OVCAR3221	PXDN	h_IPI00016112;h_	Yes	No	Yes		Yes
368	**OVCAR3368**	**SLC29A1**	**h_IPI00550382**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
148	**OVCAR3148**	**NRCAM**	**b_IPI00717140;b_**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
56	OVCAR356	FBN2	b_IPI00696214;h_	Yes	No	Yes	MEMBRANE	Yes
14	OVCAR314	LPHN2	b_IPI00687084;b_	Yes	Yes	Yes	MEMBRANE	Yes
332	OVCAR3332	CD97	h_IPI00299412	Yes	Yes	Yes	MEMBRANE	Yes
391	OVCAR3Inconsiste	CD97	h_IPI00397229	Yes	Yes	Yes	MEMBRANE	Yes
392	OVCAR3Inconsiste	CD97	h_IPI00397230	Yes	Yes	Yes	MEMBRANE	Yes
402	OVCAR3Inconsiste	CD97	h_IPI00872746	Yes	No	Yes	MEMBRANE	Yes
219	OVCAR3219	CELSR2	h_IPI00015346	Yes	Yes	Yes	MEMBRANE	Yes
200	OVCAR3200	SYPL1	h_IPI00009507;h_	Yes	Yes	Yes	MEMBRANE	Yes
245	OVCAR3245	SEMA4D	h_IPI00023807	Yes	No	Yes	MEMBRANE	Yes
216	**OVCAR3216**	**SLC39A14**	**h_IPI00014236;h_**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
254	OVCAR3254	BST2	h_IPI00026241	Yes	Yes	Yes	MEMBRANE	Yes
238	OVCAR3238	PTGFRN	h_IPI00022048;h_	Yes	No	Yes	MEMBRANE	Yes
302	OVCAR3302	GPR126	h_IPI00217481;h_	Yes	Yes	Yes	MEMBRANE	Yes
199	OVCAR3199	NT5E	h_IPI00009456	Yes	Yes	Yes	MEMBRANE	Yes
373	OVCAR3373	BMPR2	h_IPI00783156;h_	Yes	Yes	Yes	MEMBRANE	Yes
349	OVCAR3349	EMB	h_IPI00394808	Yes	No	Yes	MEMBRANE	Yes
291	OVCAR3291	LRRC8B	h_IPI00166036;h_	Yes	No	Yes	MEMBRANE	Yes
211	OVCAR3211	IFNAR1	h_IPI00012877;h_	Yes	Yes	Yes	MEMBRANE	Yes
274	OVCAR3274	SLC29A2	h_IPI00031456;h_	Yes	Yes	Yes	MEMBRANE	Yes
123	OVCAR3123	SLC12A2	b_IPI00710920;h_	Yes	Yes	Yes	MEMBRANE	Yes
180	OVCAR3180	SEL1L	h_IPI00002790	Yes	No	Yes	MEMBRANE	Yes
309	OVCAR3309	FAS	h_IPI00235003;h_	Yes	Yes	No	MEMBRANE	Yes
264	OVCAR3264	ITGB8	h_IPI00029533	Yes	Yes	Yes	MEMBRANE	Yes
163	OVCAR3163	CLDND1	b_IPI00731806;h_	Yes	No	Yes	MEMBRANE	Yes
359	OVCAR3359	KIRREL	h_IPI00470360;h_	Yes	Yes	Yes	MEMBRANE	Yes
102	OVCAR3102	ITGA9	b_IPI00706528;h_	Yes	Yes	Yes	MEMBRANE	Yes
346	OVCAR3346	NCAM2	h_IPI00376427	Yes	Yes	Yes	MEMBRANE	Yes
72	OVCAR372	NPR3	b_IPI00699662;b_	Yes	No	Yes	MEMBRANE	Yes
287	OVCAR3287	SLC2A12	h_IPI00152424	Yes	No	Yes	MEMBRANE	Yes
297	OVCAR3297	GPR107	h_IPI00184474;h_	Yes	No	Yes	MEMBRANE	Yes
105	OVCAR3105	UPK1B	b_IPI00706943;h_	Yes	No	Yes	MEMBRANE	Yes
268	OVCAR3268	MERTK	h_IPI00029756;h_	Yes	Yes	Yes	MEMBRANE	Yes
223	OVCAR3223	IGSF3	h_IPI00016890;h_	Yes	No	Yes	MEMBRANE	Yes
361	OVCAR3361	SLCO4C1	h_IPI00470538	Yes	Yes	Yes	MEMBRANE	Yes

247	OVCAR3247	IGSF9	h_IPI00024053;h_	Yes	Yes	Yes	MEMBRANE	Yes
193	OVCAR3193	SLC34A2	h_IPI00007910;h_	Yes	Yes	Yes	MEMBRANE	Yes
225	OVCAR3225	GPR110	h_IPI00017924;h_	Yes	Yes	Yes	MEMBRANE	Yes
263	OVCAR3263	SLC7A1	h_IPI00027728	Yes	Yes	Yes	MEMBRANE	Yes
270	**OVCAR3270**	**ADCY9**	**h_IPI00030099**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
275	**OVCAR3275**	**CD70**	**h_IPI00031713**	**Yes**	**Yes**	**Yes**	**MEMBRANE**	**Yes**
146	OVCAR3146	TSPAN15	b_IPI00715417;h_	Yes	Yes	Yes	MEMBRANE	Yes
149	OVCAR3149	PTPRS	b_IPI00717208;b_	Yes	Yes	Yes	MEMBRANE	Yes
356	OVCAR3356	CRB2	h_IPI00410585;h_	Yes	Yes	Yes	MEMBRANE	Yes
362	OVCAR3362	HLA-A	h_IPI00472013;h_	Yes	Yes	Yes	MEMBRANE	Yes
203	OVCAR3203	PLAUR	h_IPI00010676;h_	Yes	Yes	No		Yes
329	OVCAR3329	SLC39A6	h_IPI00298702	Yes	Yes	Yes	MEMBRANE	Yes
120	OVCAR3120	SGCE	b_IPI00709796;h_	Yes	Yes	Yes	MEMBRANE	Yes
174	OVCAR3174	ITGB6	h_IPI00000151	Yes	Yes	Yes	MEMBRANE	Yes
276	OVCAR3276	SLC26A2	h_IPI00032107	Yes	Yes	Yes	MEMBRANE	Yes
330	OVCAR3330	JAM2	h_IPI00299083;h_	Yes	Yes	Yes	MEMBRANE	Yes
66	OVCAR366	ITGA1	b_IPI00697595;b_	Yes	Yes	Yes	MEMBRANE	Yes
195	OVCAR3195	CSPG5	h_IPI00008586;h_	Yes	Yes	Yes	MEMBRANE	Yes
181	OVCAR3181	CELSR1	h_IPI00003384;h_	Yes	Yes	Yes	MEMBRANE	Yes
328	OVCAR3328	TACSTD2	h_IPI00297910	Yes	Yes	Yes	MEMBRANE	Yes
336	OVCAR3336	VTCN1	h_IPI00302614;h_	Yes	Yes	Yes	MEMBRANE	Yes
358	OVCAR3358	FOLR1	h_IPI00441498	Yes	Yes	Yes	MEMBRANE	Yes
64	OVCAR364	PTPRG	b_IPI00697151;h_	Yes	Yes	Yes	MEMBRANE	Yes
76	OVCAR376	SEZ6L2	b_IPI00699955;h_	Yes	Yes	Yes	MEMBRANE	Yes
176	OVCAR3176	ST14	h_IPI00001922	Yes	Yes	Yes	MEMBRANE	Yes
178	OVCAR3178	BCAM	h_IPI00002406;h_	Yes	Yes	Yes	MEMBRANE	Yes
192	OVCAR3192	PLXNB1	h_IPI00006644;h_	Yes	Yes	No	MEMBRANE	Yes
209	OVCAR3209	NPTN	h_IPI00011578;h_	Yes	Yes	Yes	MEMBRANE	Yes
257	OVCAR3257	ADORA1	h_IPI00026872	Yes	Yes	Yes	MEMBRANE	Yes
260	OVCAR3260	SLC3A2	h_IPI00027493;h_	Yes	Yes	Yes	MEMBRANE	Yes
298	OVCAR3298	ITGA3	h_IPI00215995;h_	Yes	Yes	Yes	MEMBRANE	Yes
372	OVCAR3372	HLA-A	h_IPI00760554;h_	Yes	Yes	Yes	MEMBRANE	Yes
81	OVCAR381	WNT5A	b_IPI00702598;h_	Yes	No	Yes	MEMBRANE	Yes
187	OVCAR3187	SLC44A1	h_IPI00005068;h_	Yes	No	Yes	MEMBRANE	Yes
229	OVCAR3229	GGT1	h_IPI00018901;h_	Yes	No	Yes	MEMBRANE	Yes
301	OVCAR3301	PPAP2C	h_IPI00216620;h_	Yes	No	Yes	MEMBRANE	Yes
343	OVCAR3343	DCBLD1	h_IPI00337612;h_	Yes	No	Yes	MEMBRANE	Yes

22	OVCAR322	SLC2A9	b_IPI00689007;h_	Yes	No	Yes	MEMBRANE	Yes
44	OVCAR344	SLITRK2	b_IPI00693299;h_	Yes	No	Yes	MEMBRANE	Yes
189	OVCAR3189	MRC2	h_IPI00005707	Yes	No	Yes	MEMBRANE	Yes
321	OVCAR3321	SLCO3A1	h_IPI00296394;h_	Yes	No	Yes	MEMBRANE	Yes
279	OVCAR3279	PLXDC2	h_IPI00044369	Yes	No	Yes	MEMBRANE	Yes
282	OVCAR3282	TM2D1	h_IPI00104219;h_	Yes	No	Yes	MEMBRANE	Yes
335	OVCAR3335	TMEM132A	h_IPI00301865;h_	Yes	No	Yes	MEMBRANE	Yes
51	OVCAR351	NTN1	b_IPI00695422;b_	Yes	No	Yes	MEMBRANE	Yes
57	OVCAR357	THBS1	b_IPI00696401;b_	Yes	No	No	MEMBRANE	Yes
250	OVCAR3250	MPZL2	h_IPI00024811	Yes	No	Yes	MEMBRANE	Yes
21	OVCAR321	ODZ3	b_IPI00688903;b_	Yes	No	Yes	MEMBRANE	Yes
278	OVCAR3278	IGSF1	h_IPI00043215;h_	Yes	No	Yes	MEMBRANE	Yes
273	OVCAR3273	FAT	h_IPI00031411	Yes	Yes	Yes	MEMBRANE	Yes
295	OVCAR3295	TMEM2	h_IPI00170706	Yes	No	Yes	MEMBRANE	Yes
240	OVCAR3240	PRNP	h_IPI00022284;h_	Yes	Yes	Yes	MEMBRANE	Yes
285	OVCAR3285	TMEM16F	h_IPI00151710	Yes	No	Yes	MEMBRANE	Yes
319	OVCAR3319	TACSTD1	h_IPI00296215;h_	Yes	Yes	Yes	MEMBRANE	Yes
269	**OVCAR3269**	**NOTCH3**	**h_IPI00029819**	**Yes**	**Yes**	**No**	**MEMBRANE**	**Yes**
87	OVCAR387	LRP1	b_IPI00704118;b_	Yes	Yes	Yes	MEMBRANE	Yes
67	OVCAR367	STX6	b_IPI00697758;h_	No	Yes	Yes	MEMBRANE	Yes
290	**OVCAR3290**		**h_IPI00154588**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
293	**OVCAR3293**		**h_IPI00168255**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
378	**OVCAR3378**		**h_IPI00787773**	**Yes**	**No**	**Yes**	**MEMBRANE**	**Yes**
374	OVCAR3374		h_IPI00783166;h_	Yes	No	Yes	MEMBRANE	Yes
367	OVCAR3367		h_IPI00514244	Yes	No	Yes	MEMBRANE	Yes